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METHODS FOR REARING *AEDES TAENIORHYNCHUS* (WIEDEMANN) IN THE LABORATORY

By Hugh R. Ford and Everett Green[1]

ABSTRACT

A regimen for rearing large and small quantities of *Aedes taeniorhynchus* (Wiedemann) is described. A single dietary source and only two feedings during larval growth are used. Since the eggs can be stored on sphagnum moss up to 3 months and hatched when needed, rearing from eggs to pupae may be scheduled to fit into 5 days, eliminating weekend work. **KEYWORDS: *Aedes taeniorhynchus*, insect egg storage, insect rearing, insect-rearing equipment, larval diets.**

INTRODUCTION

Colonization of *Aedes taeniorhynchus* (Wiedemann) was first reported by Davis (*1*)[2] and Haeger (*5*). A method for the aseptic rearing of the species was described by Nayar (*6*), and several researchers have reported other methods of rearing in the laboratory or in large flats or trays outdoors (*2, 7–10*). General rearing techniques were outlined by Gerberg (*4*). However, none of these procedures is adequate for producing large numbers of mosquitoes routinely for long periods of time. We used or modified previously described procedures to develop a method for mass rearing *A. taeniorhynchus* continuously.

REARING PROCEDURES

Adults

Holding cages 76 cm wide, 61 cm deep, and 61 cm high are maintained at 27° C and 70% relative humidity in a room with a window that provides natural light to enhance crepuscular mating. The sides and front of each cage are made of 18-mesh wire screen; the top, bottom, and back are made of plywood covered on the inside with Formica. A cloth sleeve 76 cm long is attached to a 30- by 38-cm opening in the front of the cage.

The cages are kept stocked with 4,000 to 6,000 adults of both sexes by periodically adding 1,000 to 1,500 unsexed pupae in a 354-ml waxed-paper cup. Food is provided on a 16- by 46-cm piece of nonsterile cotton that has been rolled and soaked in 10% sugar water, pressed into a 175-ml plastic cup, and hung from the ceiling of the cage. Two of these feeding wicks are used in each cage and are routinely changed on Mondays, Wednesdays, and Fridays. In addition, females are offered a blood meal each day by placing a rabbit with a closely shaved back in the cage for 3 h. A wooden stanchion constrains the rabbit by its head and neck. Sufficient rabbits are available so that no rabbit is exposed more than once every 3 days.

Eggs

The oviposition medium consists of sphagnum moss soaked for several hours in water and then pressed to dampness. It is spread loosely in enameled pans 30 cm in diameter and 10 cm high. The pans are placed in the adult cages for 1 week, then removed, covered with aluminum foil, and stored at room temperature for at least 2 weeks to insure adequate conditioning and embryonation. Because the eggs will remain viable for 2 to 3 months, it is possible to stockpile them in lieu

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[2]Italic numbers in parentheses refer to items in "Literature Cited" at the end of this publication.

of maintaining continuous daily production. However, since the foil deteriorates, it should be changed monthly.

For hatching, the eggs are first washed from the moss through a 30-mesh screen to remove most of the moss fragments. The water in the receiving pan is stirred with a circular motion that concentrates the eggs in the center of the bottom of the pan so they can be readily removed with a pipette. The pan used for hatching the eggs is 30 cm in diameter and 10 cm high. One liter of distilled water and 50 ml of a 4% solution of a commercial-grade table salt are poured in the pan. The final hatching medium contains 0.19% salt. The eggs are pipetted from the wash pan into the hatch pan and again stirred with a circular motion to concentrate the eggs and stimulate hatch. About 1 h after hatching begins, 100 mg of brewer's yeast mixed with water is gently poured into the pan. Several hours later, the eggshells and unhatched eggs are removed from the bottom of the pan with a pipette, and the larvae are left undisturbed overnight.

Larvae

For small-scale rearing we use 19- by 30- by 5-cm enameled pans with 1 liter of distilled water containing 0.19% salt (by the addition of 50 ml of 4% salt solution) and 200 mg of hog supplement (40% protein, Ralston Purina Co., ground and sifted through a 50-mesh screen). After the food is thoroughly mixed with the water, 200 to 250 first-instar larvae are added from the hatching pan. Three days later 1 g hog supplement, previously wetted with water, is added to each pan.

For larger scale rearing, we use 51- by 38- by 8-cm plastic trays (Panel Control Corp., Mt. Lakes, N.J. 07046) containing 5 liters of distilled water, 350 ml of 4% salt solution (0.28% in final solution), 2 g of hog supplement, and 2,000 to 2,500 first-instar larvae. On the third day after the larvae are introduced, 5 g of wetted hog supplement is added. To facilitate handling, the food is mixed in a slurry (1 g of food to 50 ml of water) in a plastic shaker, shaken vigorously, and poured into a plastic pitcher; a 50-ml plastic cup is used to dip the continuously agitated mixture into the trays.

Our method of obtaining reasonably accurate counts of 2,000 to 2,500 larvae involves the use of a 10-compartment, white plastic ice tray. About 200 to 250 first-instar larvae are counted into one compartment, and a similar number is estimated visually and placed in each of the other nine compartments. With experience, the desired number of larvae can be estimated with sufficient accuracy to obviate counting out the larvae in the initial compartment. The entire contents are poured into a previously prepared rearing tray. All larvae are reared in an unhumidified room at 29° C.

Pupae

Pupation begins the fifth day; however, we prefer to wait until 50% of the larvae have pupated, usually the sixth day, before collecting pupae. Pupae from each rearing tray are separated from the larvae by collecting the two stages on a screen, transferring them to a cup, and pouring them through a pupal separator (*3*). The pupae, because they are larger, are retained in the separator while the larvae pass through and are collected. Afterwards, the separator is opened and the pupae are collected. The larval collections are consolidated in the old rearing water and held one more day; any new pupae are then separated and the remaining larvae are discarded. Pupae are held in distilled water for adult emergence.

DISCUSSION

Using this procedure, one man can rear 220,000 larvae per week in only 7.5 m^2 of floorspace. Washing the eggs from the moss, setting up the hatch pans, and feeding the young larvae requires 1 h. It takes about 2 h to fill the larval-rearing trays and add the 4% salt solution and 2 g of hog supplement in slurry. An additional 2 h are needed to introduce the larvae. The supplemental feeding on the third day requires less than 1 h, and separating the pupae requires an additional 4 h. Washing the trays and returning them to the shelves for the next cycle requires approximately 2 h. Thus, the actual working time spread over 5 days is less than 14 man-hours. If the eggs are flooded on Thursdays, the trays are set up on Fridays, feeding is done on Mondays, and pupae are separated on Thursdays and Fridays, no weekend work is necessary.

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